Rat-strain dependent changes of dendritic and spine morphology in the hippocampus after cocaine self-administration

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ABSTRACT

We previously showed that cocaine self-administration increases spine density in CA1 hippocampal neurons in Lewis (LEW) but not in Fischer 344 (F344) rats. Dendritic spine morphology is intimately related to its function. Thus, we conducted a 3D morphological analysis of CA1 dendrites and dendritic spines in these two strains of rats. Strain-specific differences were observed prior to cocaine self-administration: LEW rats had significantly larger dendritic diameters but lower spine density than the F344 strain. After cocaine self-administration, proximal dendritic volume, dendritic surface area and spine density were increased in LEW rats, where a higher percentage of larger spines were also observed. In addition, we found a strong positive correlation between dendritic volume and spine morphology, and a moderate correlation between dendritic volume and spine density in cocaine self-administered LEW rats, an effect that was not evident in any other condition. By contrast, after cocaine self-administration, F334 rats showed decreased spine head volumes. Our findings suggest that genetic differences could play a key role in the structural plasticity induced by cocaine in CA1 pyramidal neurons. These cocaine-induced alterations could be related to differences in the memory processing of drug reward cues that could potentially explain differential individual vulnerability to cocaine addiction.

INTRODUCTION

Addiction is a complex and chronic neuropsychiatric disorder characterized by a long-term propensity for relapse. It has been suggested that, following drug exposure, persistent maladaptive memories take over the learning and plasticity processes normally involved in associations between environmental stimuli and natural reinforcers, and these processes seem to be of fundamental importance underpinning drug-seeking relapse (Milton & Everitt 2012). Long-term behavioral changes that occur as a result of experience are thought to depend on the formation and reorganization of synaptic connections (structural plasticity) in distinct brain circuits. Thus, the study of the effects of cocaine on structural plasticity may help to better understand the neurobiological basis of cocaine addiction.

Dendritic spines (for simplicity, spines) are the sites of most excitatory synapses in the brain, and they are considered key elements in learning and memory (Kasai *et al.* 2010; Yuste 2010). Provided that the majority of spines have been shown to have synaptic contacts (Arellano *et al.* 2007b), the quantification and analysis of these structures are highly appropriate to identify possible alterations in brain circuitry. Several studies have demonstrated that abused drugs lead to modifications in spines in different cortical and subcortical regions

(Robinson & Kolb 2004; Ballesteros-Yanez *et al.* 2007b; Ballesteros-Yanez *et al.* 2007a; Ballesteros-Yanez *et al.* 2008; Russo *et al.* 2010; Dumitriu *et al.* 2012; Miguens *et al.* 2015). Most of the studies have focused on the nucleus accumbens and prefrontal cortex given the importance of the mesocorticolimbic reward system in addiction. However, several recent reports suggest that a broader circuit is involved in cocaine addiction, including structures like the amygdala and hippocampus (Koob & Volkow 2010). It has also been reported that addictive drugs can alter adult neurogenesis in the hippocampus (Canales 2007), and other authors suggest that hippocampal neurogenesis protects against cocaine-primed relapse (Deschaux *et al.* 2012). The activation of this structure is involved in the reinstatement of cocaine seeking (Vorel *et al.* 2001), and damage to the ventral subiculum of the hippocampus was shown to reduce cocaine self-administration in rats (Caine *et al.* 2001). Moreover, the dorsal and ventral hippocampus have been involved in the association between cocaine and contexts, as well as in cue-induced and cocaine-primed reinstatement (Fuchs *et al.* 2005; Rogers & See 2007; Lasseter *et al.* 2010). In addition, decreased glutamate transporter binding and long-term potentiation (LTP) facilitation in the CA1 field of the hippocampus after cocaine self-administration was observed (Del Olmo *et al.* 2006; Miguens *et al.* 2008; see also Thompson *et al.* 2004)—an effect that seems to be dependent on dopamine and metabotropic glutamate receptors (Fole *et al.* 2014).

We previously reported that Lewis (LEW) rats are more sensitive than Fischer 344 rats (F344) to the reinstating effects of cocaine after setting similar cocaine self-administration (Miguens *et al.* 2013)—two strains that have been proposed as a useful model to study genetic vulnerability to drug addiction (Kosten & Ambrosio 2002). In addition, we found strain-specific differences in cocaine-induced neuroplasticity in these two strains of rats in the hippocampus. We observed impaired LTP depotentiation in the F344 strain—a result that is only found in LEW rats after cocaine self-administration (Miguens *et al.* 2011). Furthermore, F344 rats showed higher spine density than LEW rats, and cocaine self-administration increased spine density in the LEW strain—no increase was observed in the F344 (Miguens *et al.* 2015). Thus, these differences in cocaine-induced neuroplasticity could reflect differences in neuronal structure and function in these strains of rats.

It has been suggested that morphological changes of spines may represent significant modifications in their biochemical function. There is also a correlation between several morphological parameters, such as the postsynaptic density size, spine head volume and the number of vesicles in presynaptic terminals, in many brain regions including the neocortex, hippocampus and cerebellum (Harris & Stevens 1988, 1989; Tashiro & Yuste 2003), indicating that changes in spine morphology are related to functional modifications in the mechanisms of information processing at spine level (Yuste 2010). Given that the hippocampus has been involved in drug context memory formation (Koob & Volkow 2010), ascertaining whether or not there are alterations in the neuroplasticity of this structure is imperative to understand more about the mechanisms that underlie drug-associated memory formation and its relationship to relapse to cocaine seeking. Newly generated synaptic inputs distributed along the dendrites might clarify how information is integrated into the neurons to produce their own output signals after cocaine exposure.

Therefore, following our previous work demonstrating strain-specific changes in spine density in the hippocampus after cocaine self-administration (Miguens *et al.* 2015), here, we have studied in detail the morphological changes at the dendrite and spine level, in the CA1 collaterals of apical dendrites of the hippocampus of LEW and F344 rats.

MATERIAL AND METHODS

Animals

We analyzed the dendrites of 23 adult male LEW ($n = 12$) and F344 ($n = 11$) rats (Harlan Interfauna Ibérica, Barcelona, Spain) from a previous study (Miguens *et al.* 2015). The animals were experimentally naïve and weighed 275–350 g prior to the initiation of the experiments. They were singly housed in a climate-controlled room (23°C) with a 12-hour light–dark cycle (0800–2000 lights on) with free access to Purina laboratory feed (Panlab, Barcelona, Spain) and tap water. All animals were maintained and handled according to European Union guidelines for the care of laboratory animals (Directive 2010/63/EU), and we followed the 'Principles of Laboratory Animal Care'.

Catheter surgery

As previously described (Miguens *et al.* 2013), subjects were implanted with intravenous polyvinylchloride tubing in the jugular vein (0.064 i.d.) under ketamine (40 mg/kg) and diazepam (10 mg/kg) anesthesia approximately at the level of the atrium. Rats were allowed to recover for 7 days after surgery, and catheters were flushed daily with 0.5 ml of a solution of antibiotic (gentamicin, 0.10 mg/ml) dissolved in heparinized saline to prevent infections and to maintain catheter patency. At the end of the experiments, the catheter patency was

tested with the barbiturate anesthetic thiopental (10 mg/kg, i.v.), and it was assumed to remain unblocked if the rat immediately lost consciousness.

Experimental procedure

Behavioral experiments were performed in 12 operant conditioning chambers (Coulburn Instruments, Allentown, PA, USA) with two fixed levers. Active lever presses resulted in cocaine infusions, while inactive lever presses were recorded but had no programmed consequences. Before surgery, rats were food deprived to 95 percent of their free-feeding weight, and they were subjected to a fixed ratio (FR) 1 schedule of food reinforcement over several sessions until they showed stable operant behavior. Then, they were allowed *ad libitum* access to food, and surgery was performed. After a postoperative recovery period of at least 7 days, the rats were food deprived again to 95 percent of their free-feeding body weight. Subsequently, they were trained to self-administer cocaine (1 mg/kg per infusion; LEW and F344, $n = 6$ in each strain) or saline (LEW $n = 6$, and F344 $n = 5$) in 100 µl of volume over 10 seconds for at least 20 days under an FR1 schedule of reinforcement with a timeout period of 10 seconds. A stimulus light over the lever signaled drug availability and was switched off during drug infusion and the timeout period. Daily training sessions lasted 2 hours or until 20 cocaine infusions had been earned. A microliter injection pump (Harvard 22; Harvard Apparatus, Holliston, MA, USA) was used to deliver the cocaine or saline infusions over 10 seconds when the animals pressed the active lever.

Intracellular injections

Single-cell microinjections were performed according to the methods described in our previous study (Miguens *et al.* 2015; for further methodological details, see Benavides-Piccione *et al.* 2013; Elston & Rosa 1997). Briefly, 24 hours after the last self-administration session, rats were intracardially perfused with 4 percent paraformaldehyde in phosphate buffer (PB; 0.1 M; pH 7.3), and their brains were removed and immersed in 4 percent paraformaldehyde in PB for a further 24 hours. Coronal sections (200 µm) were obtained with a vibratome (Lancer 1000 vibratome; St. Louis, MO, USA) at the level of the dorsal hippocampus. CA1 hippocampal pyramidal cells were individually injected with Lucifer yellow (8 percent in 0.1 M Tris buffer, pH 7.4) by continuous current until the distal tips of the collateral branches of the apical dendrites and the dendritic spines were readily visible. Then, sections were processed with a rabbit antibody against Lucifer yellow (1:400 000 made in the Cajal Institute) and a secondary antibody against rabbit conjugated with Alexa 488 (1:1000; Invitrogen, Carlsbad, CA, USA). Sections were mounted and coverslipped using ProLong® Gold antifade reagent (Invitrogen).

Morphometric analysis of spines

3D z-stack images were taken for analysis with Zeiss confocal equipment (LSM 710, equipped with an Axio Observer. Z1 inverted microscope; Carl Zeiss MicroImaging GmbH, Germany), using a $0.057 \times 0.057 \times 0.14\ \mu m^3$ voxel size. A 63× immersion objective was used (Zeiss Objective Plan-Apochromat 63×/1.40 NA Oil DIC M27) with a theoretical minimum resolvable resolution in the x–y plane of about 0.14 µm. We collected at least eight CA1 hippocampal collateral dendrites (Fig. 1) from each animal and made sure that each dendrite was acquired from the apical shaft to the end of its distal tip. This procedure usually rendered three different files for a single dendrite. Images were deconvolved using AUTODEBLUR software (MediaCybernetics, Inc.; Bethesda, MD, USA) to decrease the blur around spines, and stacks were integrated into a single volumetric dataset with VIAS software (Computational Neurobiology and Imaging Center; Mt Sinai School of Medicine, New York, NY, USA). Tridimensional reconstruction of the spines in the collateral branches of the apical dendrite was performed using the semi-automated software NEURONSTUDIO (http://research.mssm.edu/cnic/tools-ns.html), and a dynamic threshold was used to adjust the local intensity distribution of the data. The attach ratio value used for the neurite endpoints of the model was 3, and to control the model vertices, we used a discretization ratio of 0.5. Regarding spines, the minimum and maximum values for height were 0.2 and 4.0 µm, respectively, with a maximum width value of 3 µm. The maximum number of voxels was 10 for stubby spines and 5 in the case of non-stubby spines. A human operator, blinded to the condition, selected 5–7 apical collateral dendrites from different neurons of each animal ($n = 5$–6 per group), and several morphological parameters were measured:

- averaged dendritic diameter,
- dendritic volume as a function of the distance from the apical trunk,
- dendritic surface area as a function of the distance from the apical trunk,
- spine density,
- spine head volume (rayburst sampling algorithm),
- spine head diameter and
- spine length (distance from the tip of the spine to the surface of the model; this value is an approximate measure of the length of the spine).

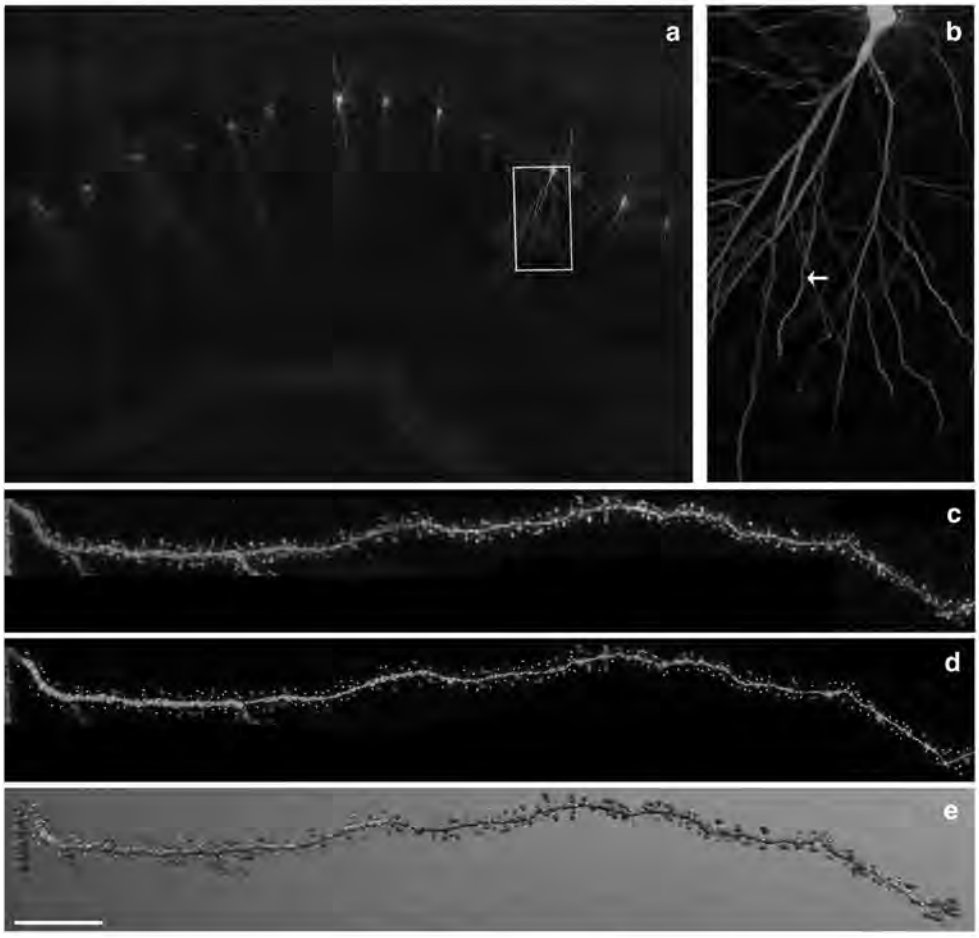

Figure 1 Tridimensional reconstruction of dendrites and spines of CA1 pyramidal cells. (a) Confocal projection image from a panoramic view of CA1 pyramidal neurons injected with Lucifer yellow. (b) Magnification of the portion of the injected pyramidal cell boxed in panel a. (c) Higher magnification of the apical collateral dendrite indicated with an arrow in panel b. (d) Reconstruction of the dendritic process and spines with NEURONSTUDIO software. (e) 3D view of the dendrite reconstructed in panel d. Scale bar = 275 μm in panel a, 60 μm in panel b and 10 μm in panels c, d and e

Additionally, we used the spine classification provided by the NEURONSTUDIO software, which assigns each particular spine to one of the three major classes ('mushroom', 'thin' and 'stubby'). Spines with a head-to-neck-diameter ratio greater than 1.1 μm and a head diameter equal or greater than 0.35 μm were classified as mushroom subtype; spines with a head-to-neck-diameter ratio greater than 1.1 μm and a head diameter lower than 0.35 μm were classified as thin subtype; and spines with a head-to-neck-diameter ratio lower than 1.1 μm were classified as stubby subtype.

Statistical analysis of the data

Averaged dendritic diameter was analyzed by means of a two-way ANOVA with *strain* and *treatment* as independent variables. Dendritic volume, dendritic surface area and dendritic surface-to-volume ratios were analyzed using a mixed ANOVA with *strain* and *treatment* as independent variables. The within-subject distance to the apical dendrite shaft was measured, and ANOVA was performed. The morphometric parameters of the spine (head volume, head diameter and length) were analyzed using a two-way ANOVA with *strain* and *treatment* as independent variables. In addition, these parameters were analyzed as a function of the distance to the apical trunk (subdivided into consecutive 10-μm segments) adding the within-subject factor *distance to the apical dendrite shaft*. Two-way ANOVA followed by one-way ANOVAs was performed to analyze interaction effects. Cumulative frequencies were compared across groups using, first, the normal distribution Kolmogorov–Smirnov (K–S) fitting test and then K–S two-sample tests for subsequent paired comparisons. Correlation analysis between the different parameters quantified was performed using Spearman analysis. Values of $P < 0.05$ were considered as significant. Post hoc comparisons were performed by using

Bonferroni corrections when appropriate. All statistical analyses were performed using the SPSS statistical package (version 19.0).

RESULTS

Cocaine self-administration

Cocaine self-administered rats of both strains clearly showed a higher number of infusions than saline self-administered rats ($F_{1,\ 19} = 56.93$; $P < 0.0001$). However, there were no statistically significant differences in the total cocaine consumption between these two strains of rats after 20 days of cocaine self-administration (221.8 ± 26.05 mg/kg for LEW and 242.2 ± 28.22 mg/kg for F344; $t_{10} = 0.53$, $P = 0.61$). A detailed description of the behavioral results can be found in Miguens *et al.* (2015).

Morphological analysis of collateral dendrites of hippocampal CA1 pyramidal cells

We analyzed 5–7 whole dendrites per animal from 3D confocal images using NEURONSTUDIO software. The following parameters were obtained: averaged dendritic diameter of the whole dendrite, dendritic volume and surface as a function of the distance to the apical trunk, and the dendrite surface-area-to-volume ratio (Fig. 2).

Strain differences in averaged dendritic diameter

When we analyzed the averaged dendritic diameter, the ANOVA revealed a significant main effect for *strain* (Fig. 2a; $F_{1,\ 19} = 7.80$, $P < 0.05$); LEW rats showed larger dendritic diameters than F344 rats. However, no differences in the results of the *treatment* or the *strain* × *treatment* interaction were observed: i.e. cocaine self-administration did not affect averaged dendritic diameters in either F344 or LEW rats.

Cocaine self-administration increases dendritic volume and dendritic surface area in LEW but not in F344 rats

We analyzed dendritic volume and dendritic surface area as a function of the distance from the apical trunk. In line with previous observations (Bannister & Larkman 1995), in the present study, dendritic volume ($F_{11,\ 176} = 139.18$, $P < 0.001$) and dendritic surface area ($F_{11,\ 176} = 79.37$, $P < 0.001$) decreased along the dendrite as a function of the distance to the apical trunk.

The mixed ANOVA revealed a *distance* × *strain* × *treatment* interaction ($F_{11,\ 176} = 2.75$, $P < 0.01$) in dendritic volume and a trend bordering on significance in surface area ($F_{11,\ 176} = 1.67$, $P = 0.084$). We found a significant enlargement in the volume ($F_{11,\ 88} = 3.20$, $P < 0.01$) and the surface area ($F_{11,\ 88} = 3.18$, $P < 0.01$) of the

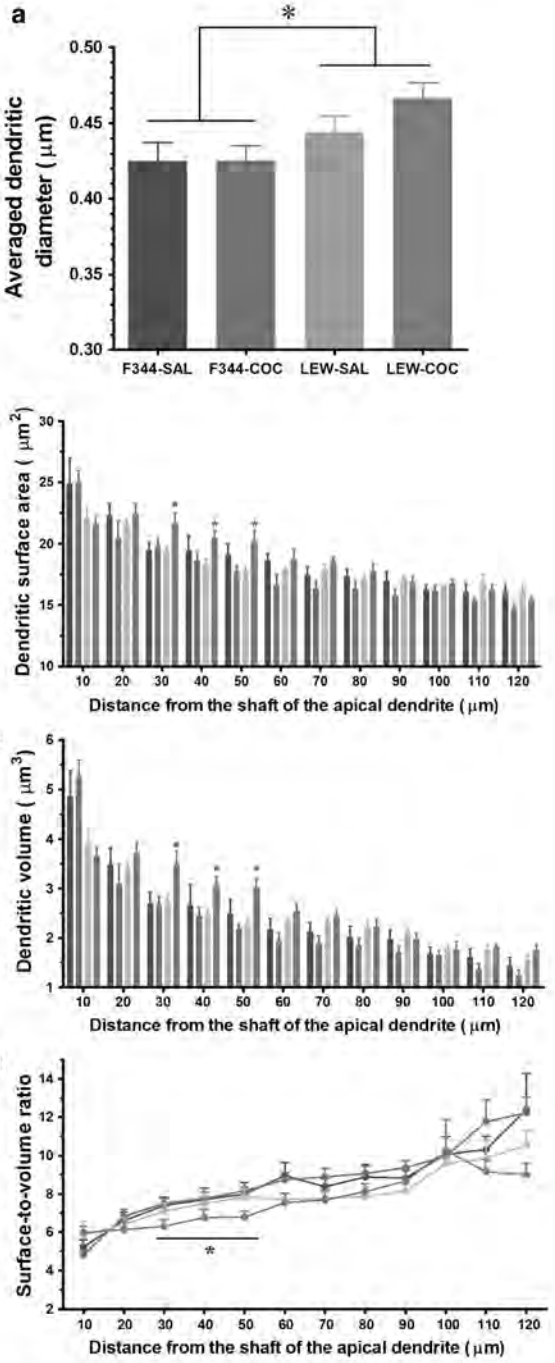

Figure 2 Analysis of the different morphological parameters of the dendritic processes. LEW rats showed larger averaged dendritic diameters than F344 rats ($P < 0.05$), but no statistical differences were observed as a consequence of cocaine treatment (a). Cocaine self-administration was related to increased dendritic surface area (b) and volume (c), and decreased surface-area-to-volume ratio (d) in the proximal part of the dendrite (30, 40 and 50 μm) in LEW but not in F344 rats. Data showed the mean ± standard error of mean. *$P < 0.05$ LEW-COC with respect to LEW-SAL; $n = 5$–6 animals per group.

dendrite as a function of the distance from the apical trunk, in cocaine self-administered LEW rats compared with their saline self-administered counterparts. Significant differences were observed at 30, 40 and 50 μm from the shaft of the apical dendrite (Fig. 2b & c). However, no differences were observed in the F344 strain as a consequence of cocaine self-administration. In addition, the surface-area-to-volume ratio was also altered in this part of the dendrite in LEW cocaine self-administered rats (Fig. 2d; $F_{1,\ 30} = 7.67$; $P < 0.05$); it was decreased in comparison with their saline counterparts. Representative projected z-stacks reconstructed by means of NEURONSTUDIO from each experimental condition are shown in Fig. 3.

Analysis of spine density and morphology

The main morphometric parameters for spines were studied in detail to determine whether the different

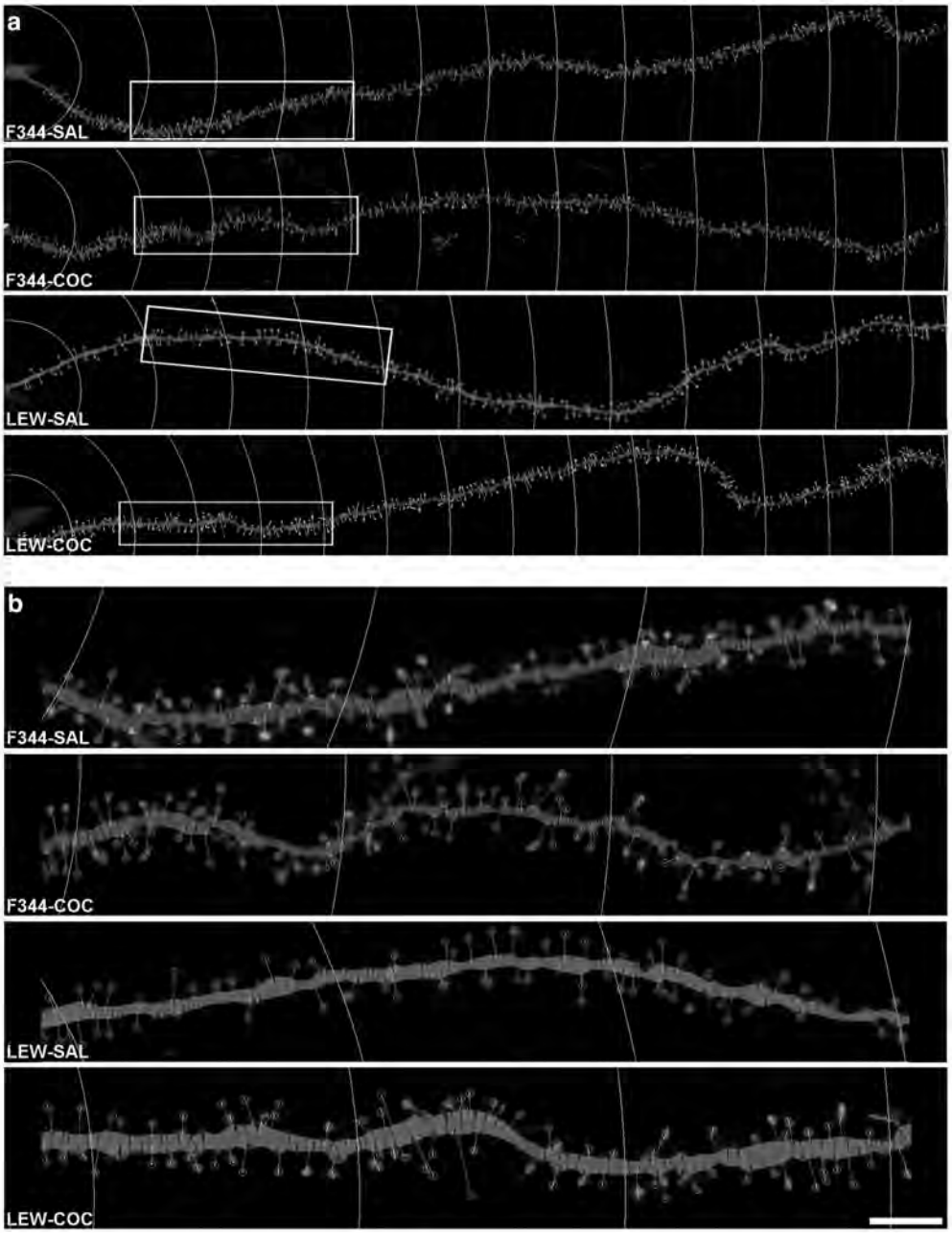

Figure 3 Dendritic reconstruction using NEURONSTUDIO software. (a) Projections of the reconstructions of high-resolution 3D confocal stacks that illustrate the representative dendritic volume and surface area for each experimental condition. (b) Higher magnification image of the dendritic segments within the boxed area in panel a (dendritic fragments spanning the 30-, 40- and 50-μm Sholl spheres from the shaft of the apical dendrites). Automatically identified spines are depicted in green, dendritic surface is depicted in red and spheres for Sholl analysis are shown as white lines. Scale bar: 10 μm in panel a and 2.5 μm in panel b

experimental conditions yielded different morphological profiles (Fig. 4). We first analyzed spine density and spine size, both as average measurements in the whole dendrite and as function of the distance along the collateral dendrite. Spine size was characterized by three different parameters: spine length, spine head volume and spine head diameter (which is partly related to the spine head volume).

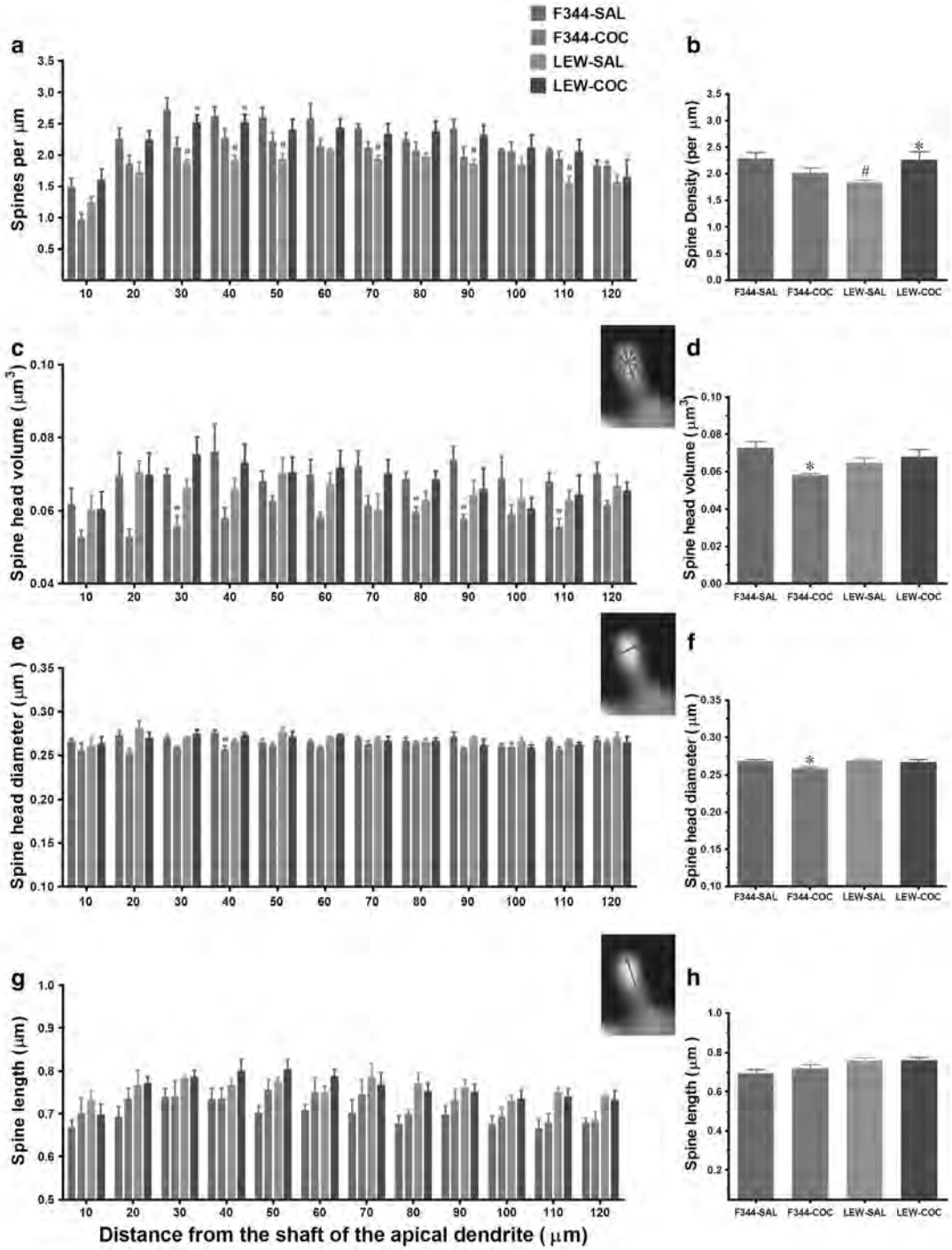

Figure 4 Analysis of spine density and spine morphology. The different morphological parameters analyzed in each experimental condition are shown: spine density (a and b), spine head volume (c and d), spine head diameter (e and f) and spine length (g and h). The panels on the left show the values as a function of the distance to the apical dendritic trunk, and the panels on the right show the same parameters averaged in the whole dendrite. Graphs show the mean ± standard error of mean. *$P < 0.05$ and **$p < 0.01$ denote a significant difference with respect to saline group; #$P < 0.05$ denotes a significant difference with respect to F344 saline group $n = 5$–6 animals per group

Spine density

As expected from our previous observations (Miguens *et al.* 2015), the ANOVA revealed a significant *strain* × *treatment* interaction effect in averaged spine density (Fig. 4b; i.e. spine density measured along the whole dendrite; $F_{1,\ 19} = 9.72$, $P < 0.01$). With respect to strain differences, F344 rats showed higher spine density than LEW rats (F334-SAL versus LEW-SAL). In addition, cocaine self-administration induced a slight but significant increase in averaged spine density in LEW rats. However, no significant differences were observed between saline and cocaine F344 rats. The mixed repeated measures ANOVA also showed a significant *distance* × *strain* × *treatment* interaction effect ($F_{11,\ 209} = 2.58$, $P < 0.01$). Increased spine density in LEW after cocaine self-administration seemed to be due to differences in the proximal rather than in the terminal portion of the dendrite (Fig. 4a).

In our previous study using manual counting by means of NEUROLUCIDA software (MicroBright-Field, Inc., Vermont, USA), we also found underlying differences between LEW and F344 rats and that cocaine self-administration increased spine density in LEW but not in F344 rats (Miguens *et al.* 2015). Here, we used the semi-automated software NEURONSTUDIO (http://research.mssm.edu/cnic/tools-ns.html) and found similar results. Focusing on analyzing spine morphology, a human operator, blinded to the condition, verified and manually corrected any errors in spine identification using a conservative criterion (i.e. no visible necks connected to the dendritic shaft or spine necks connecting with another dendritic process) to eliminate uncorrected spines without adding new spines. Thus, the number of spines counted in the present study was lower (25.3 percent less) than in the former one, but the same pattern of changes was found. It is important to note that analyzing the 'raw' data generated by NEURONSTUDIO (i.e. without removing those spines with no visible necks), we have obtained a difference of approximately 8.6 percent in spine density between studies (data not shown). This difference is in accordance with previous reports that showed less spine detection by NEURONSTUDIO software compared with manual detection results by a neurobiology expert (Shi, Huang & Hong 2014).

Spine morphology (size)

Among the parameters describing spine size, the analysis of spine head volume (Fig. 4c & d) revealed a significant *strain* × *treatment* interaction effect ($F_{1,\ 19} = 9.10$, $P < 0.01$); F344-COC showed reduced spine head volumes compared with F344-SAL ($P < 0.05$). In turn, the Sholl analysis also revealed a significant main effect in the *strain* × *treatment* interaction ($F_{1,\ 19} = 6.31$, $P < 0.05$); LEW-SAL showed lower spine head volumes than F344-SAL ($P < 0.05$). With regard to spine head diameters (Fig. 4e & f), the ANOVA revealed a significant *treatment* effect ($F_{1,\ 19} = 4.60$, $P < 0.05$), with F344-COC showing reduced spine head diameters with respect to F344-SAL ($P < 0.05$). Finally, regarding spine length (Fig. 4g & h), the ANOVA revealed a main effect of *strain* (Fig. 4h; $F_{1,\ 19} = 8.85$, $P < 0.01$); LEW rats showed longer averaged spine length than F344 rats. Supporting information Figures S1–S3 show data concerning spine density, spine head volume, spine head diameter and spine length for each of the spine subtypes classified (stubby, Figure S1; thin, Figure S2; and mushroom, Figure S3).

To investigate this effect further, we then analyzed the frequency distribution of spine head volume, spine head diameter and spine length (Fig. 5). There were significant differences in the frequency distribution of spine head volumes: Larger spine heads and longer spines were more frequent in LEW cocaine self-administered rats than in the corresponding saline animals (Fig. 5a). By contrast, F344 cocaine self-administered rats showed a lower frequency of spines with larger head volume and head diameter (Fig. 5a & c). A higher frequency of shorter spines and a lower frequency of longer spines were observed as compared with F344 self-administered (Fig. 5e). K–S goodness-of-fit test values are depicted in Table 1.

Correlation analyses between dendritic volume and the various spine morphological parameters analyzed

We then examined whether there was a potential correlation between dendritic volume in the proximal segment of the dendrite and the different spine morphological parameters analyzed in the present study (Fig. 6). As shown in the figure, in LEW cocaine self-administered rats, dendritic volume was positively correlated with spine densities (Fig. 6b) and spine head volumes (Fig. 6d) and diameters (Fig. 6f), and negatively correlated with spine neck length (Fig. 6h). However, we did not find any correlation between these parameters in saline self-administered LEW rats (Fig. 6b, d, f & h), or in either saline or cocaine self-administered F344 rats (Fig. 6a, c, e & g).

DISCUSSION

We have reported here underlying strain differences in the morphology of the collateral dendrites in the stratum radiatum of the CA1 pyramidal cells, as well

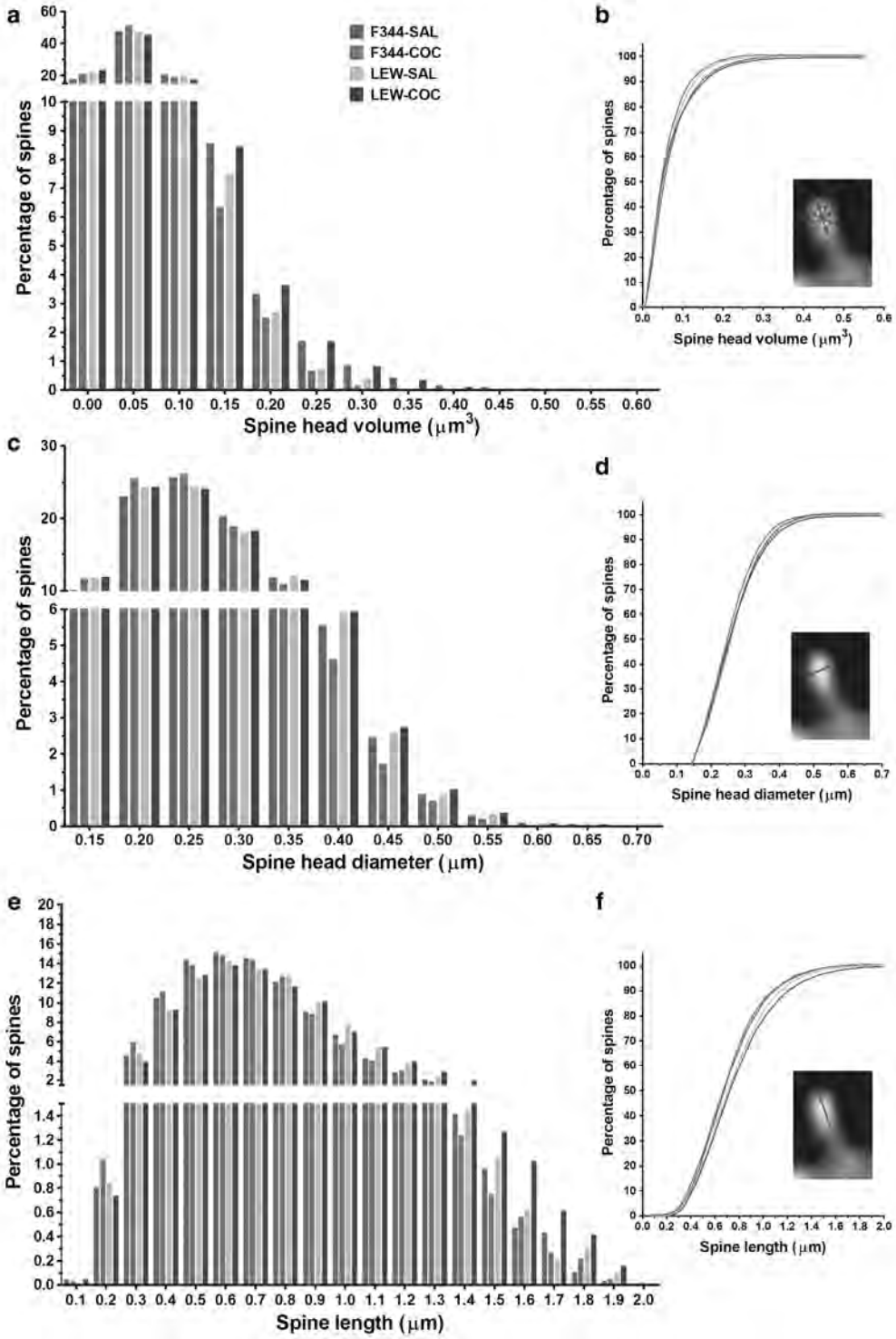

Figure 5 Frequency distributions of spine head volume, spine head diameter and spine length. Panels show frequency distribution histograms (a, c and e) and cumulative distribution functions as a percentage (b, d and f) of the morphological parameters analyzed: spine head volume (a and b), spine head diameter (c and d) and spine length (e and f). LEW cocaine self-administered rats compared with their saline self-administered counterparts showed a higher percentage of spines with larger spine head volume (0.15 to 0.45 μm^3 intervals) and length (1.4 to 1.9 μm intervals). By contrast, compared with their saline self-administered counterparts, F344 cocaine self-administered rats showed a lower percentage of spines with larger spine head volume (0.15 to 0.45 μm^3 intervals), lower percentage of spines with lower spine head diameter (0.05 to 0.10 μm intervals) and lower percentage of longer spines (1.0 to 1.7 μm intervals)

Table 1 Kolmogorov–Smirnov goodness-of-fit test for the dendritic spine parameters analyzed.

		Goodness-of-fit statistics Kolmogorov–Smirnov
Spine head volume	F344-SAL versus F344-COC	0.075, $P < 0.0001$
	LEW-SAL versus LEW-COC	0.041, $P < 0.0001$
Spine head diameter	F344-SAL versus F344-COC	0.050, $P < 0.001$
	LEW-SAL versus LEW-COC	0.007, $P = 0.983$
Spine length	F344-SAL versus F344-COC	0.024, $P = 0.009$
	LEW-SAL versus LEW-COC	0.029, $P = 0.002$

F344 = Fischer 344; LEW = Lewis.

as strain-specific effects of cocaine self-administration. A schematic summary of the results is presented in Fig. 7. With respect to strain-based morphological differences, we found that LEW rats had significantly greater dendritic diameters and longer spines but lower spine density compared with the F344 strain (Fig. 7, bottom). Moreover, the analysis revealed that cocaine self-administration increased volume and surface area in the proximal portion of the dendrite in LEW but not in F344 rats. Furthermore, in LEW cocaine self-administered rats, dendritic volume in this part of the dendrite was positively correlated with spine density, spine head volume and spine head diameter, and inversely correlated with spine length. When we analyzed frequency distributions in the entire dendrite, a higher percentage of spines with larger head volume and length were found in LEW rats subjected to cocaine self-administration as compared with their saline counterparts (Fig. 7, right). Finally, we have observed a reduction in spine head volume and spine head diameter in F334 cocaine self-administered rats compared with their saline counterparts—an effect that was mainly related to the 'stubby' and 'thin' subtypes (Fig. 7, left).

Strain-based structural plasticity differences

Strain-based structural plasticity differences in CA1 pyramidal neurons may be implicated in the different responses that LEW and F344 rats display to cocaine and other drugs of abuse. In the most of mammalian forebrain structures, pyramidal neurons are found and have been associated with sophisticated cognitive functions. Although pyramidal cells have common features, differences in neuron structure have been described among different regions of the brain and different species (Elston 2000; Jacobs *et al.* 2001; Ballesteros-Yáñez *et al.* 2006; Benavides-Piccione *et al.* 2006; Elston *et al.* 2011; Oga *et al.* 2013; Elston & Manger 2014), and the differences in their structure are thought to underpin functional differences (see Elston 2003; Jacobs & Scheibel 2002; Spruston 2008; Elston & Fujita 2014 for reviews). We previously reported strain-specific differences between LEW and F344 rats in different brain areas including the hippocampus (Ballesteros-Yanez *et al.* 2007a; Ballesteros-Yanez *et al.* 2008; Miguens *et al.* 2015). Our present results showed that averaged dendritic diameter in LEW rats was larger than in the F344 strain (Fig. 2a). Dendritic diameters are associated with cable properties (Rall 1995), and the differences in the dendritic diameter likely affect the physiological activity of the neurons given that dendrite diameter is closely related to electrical conductivity (Holmes 1989; Mainen & Sejnowski 1996; Vetter, Roth & Hausser 2001). It may be that the decreased averaged diameter of the dendrites in the F344 strain is compensated by the increased spine density that this strain exhibits as compared with the LEW strain. Thus, these structural differences could be related to differences in neuron conductivity and function, and this could be an explanation for the better performance in the radial maze that LEW rats display compared with the F344 strain (Fole *et al.* 2011). As we have previously shown, LTP depotentiation is impaired in the F344 strain (Miguens *et al.* 2011), perhaps as a consequence of the high spine density and/or the shorter spines in this strain under basal conditions.

Strain-specific cocaine-induced effects on compartment-specific plasticity

To assess the effects of cocaine self-administration on the morphological characteristics of the dendritic processes, we have analyzed the volume and surface area of the dendrite as a function of the distance to the apical trunk. We detected that these dendritic parameters are increased at the segments comprised between 30 and 50 µm from the apical trunk in LEW cocaine self-administered animals when compared with their saline self-administered counterparts (Fig. 2b and c). However, no differences were found in F344 rats as a consequence of cocaine treatment. This suggests that structural plasticity in LEW rats is more sensitive to cocaine effects than in the F344 strain, which seems to be more resistant. The

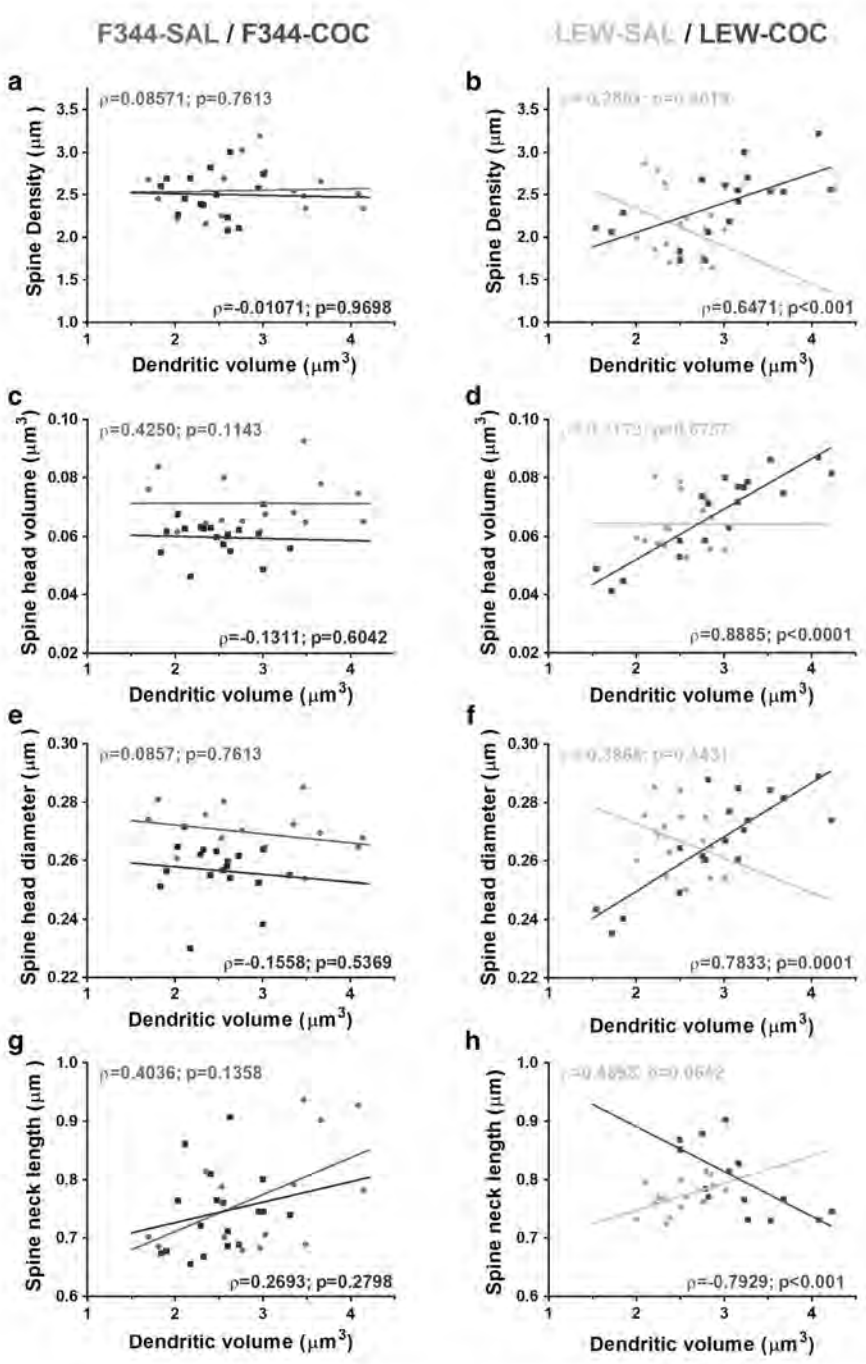

Figure 6 Correlation analyses between dendritic and spine morphology in the proximal part of the dendrite. The correlations between dendritic volume and the different parameters of spine morphology analyzed are shown for F344 (saline, green dots; cocaine, blue dots) and LEW rats (saline, orange dots; cocaine, red dots). Significant correlations were classified as weak [Spearman's rho (ρ) value lower than 0.40], moderate ($0.4 < \rho < 0.7$) and strong ($\rho > 0.7$). Points represent the averaged values of 5–7 dendrites obtained in each animal over a distance spanning 30, 40 and 50 μm from dendritic shaft of the apical dendrite ($n = 15$–18 distance points).

peculiar correlations between the different parameters of spine size and dendritic volume in the dendrite proximal segments were only found after cocaine self-administration in the LEW strain, and they were not evident in any other condition. This result could be indicative of a spino-dendritic coupling that occurs as a consequence of cocaine treatment in this strain. It has been suggested that spino-dendritic cross-talk could be implicated in long-lasting Ca^{2+} transients that occur simultaneously in neighboring spines. This results in overlap of the microdomains, providing a coincidence detection mechanism for metabotropic glutamate receptor-mediated activity (Schmidt *et al.* 2007). With regard to this, it has recently been

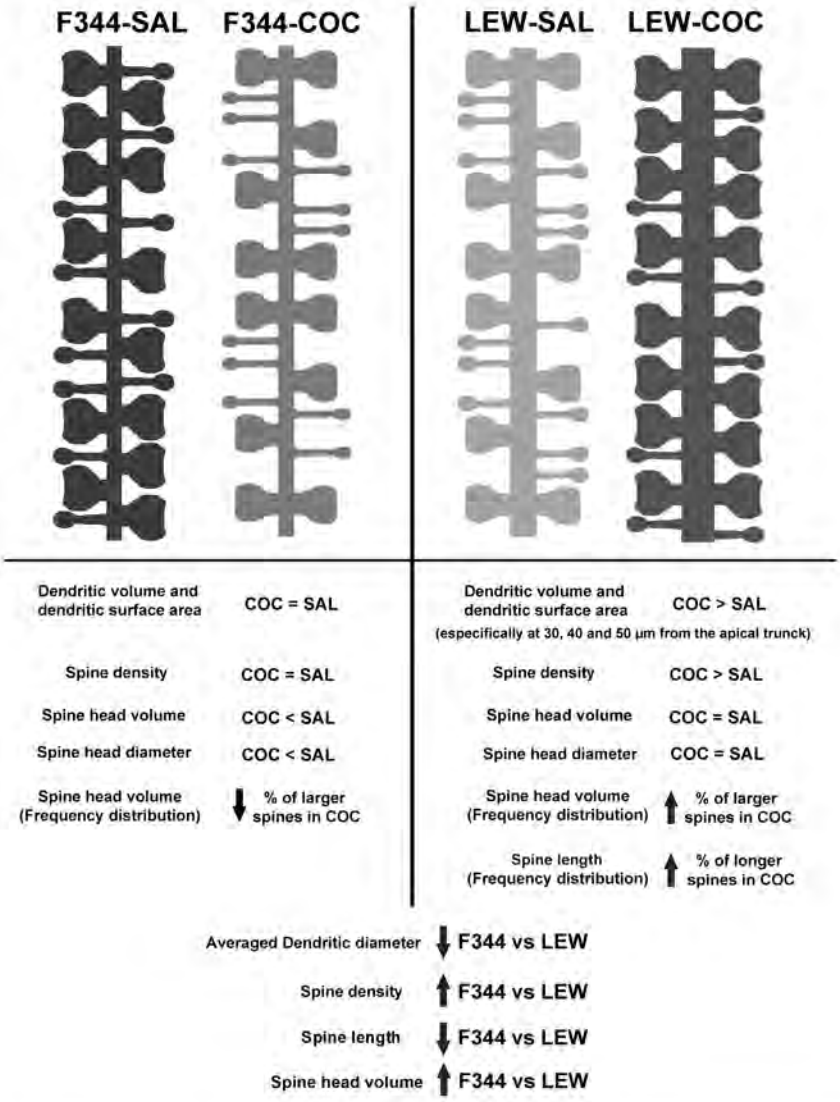

Figure 7 Schematic diagram and summary of the main results. The figure illustrates the more important morphological strain differences before and after cocaine self-administration in CA1 dendrites and spines. As we stated in the Introduction section, it is important to note that LEW rats are more sensitive to the reinstating effects of cocaine after setting similar cocaine self-administration than F344 rats (Miguens *et al.* 2013)

shown that metabotropic glutamate receptors play a key role in protein synthesis-dependent LTP cocaine-induced facilitation in the LEW strain (Fole *et al.* 2014).

The analysis of cocaine self-administration effects in the averaged spine head volume, head diameter and spine length in the whole dendrite revealed an intriguing result: Spine head volume and diameter in F344 rats were decreased after cocaine self-administration, a reduction that seems to be especially pronounced in the 'stubby' and 'thin' spine subtypes. It has been reported that induction of hippocampal LTD resulted in decreased spine head volume and diameter and that decreased spine head volume correlates with decreased synaptic strength (Okamoto *et al.* 2004; Zhou, Homma & Poo 2004). Thus, this decrease in spine head volume in F344 cocaine self-administered rats could be involved in the different sensitivity to cocaine-induced reinstatement (Miguens *et al.* 2013) that is shown by this 'addiction-resistant' strain with respect to the LEW 'addiction-prone' strain.

Subsequently, we analyzed these parameters based on the frequency distribution of spines in the entire dendrite. We observed that cocaine self-administered LEW rats have a higher percentage of spines with larger head volume as well as a higher percentage of longer spines compared with LEW saline animals —values similar to F344 saline self-administered rats. By contrast, in F344 rats, the reduction in spine head volume after cocaine self-administration (Fig. 4d) was related to a lower percentage of larger spines. These changes could be also related to functional considerations: Spine volumes are proportional to the areas

of postsynaptic densities, and spine head volume is directly related to the strength of synaptic currents (Harris & Stevens 1989; Schikorski & Stevens 1997; Arellano *et al.* 2007a). Thus, an increased frequency of spines with larger head volume in the LEW strain after cocaine self-administration would be expected to be related to augmented synaptic strength. According to Matsuzaki *et al.* (2004) and Kasai *et al.* (2010), small dendritic spines are preferential sites for long-term potentiation induction, whereas large spines might represent physical traces of long-term memory. Thus, our results suggest a possible role of dendritic volume as a regulatory factor in the actions that cocaine could have in context memories during cocaine self-administration in the LEW strain. In this regard, it has been suggested that the alteration of the surface-to-volume ratio of the dendrite could have a pronounced effect on the local amplitude of chemical signals (Helmchen 2008). As seen in the present study, this ratio was altered in cocaine self-administered LEW rats in the proximal segment of the dendrite (Fig. 2d). It is possible that the increase in the dendritic volume and spine density after cocaine self-administration in the LEW strain was also related to enhanced conductivity in CA1 hippocampal neurons. This would be in agreement at the physiological level with the LTP cocaine-induced facilitation that was observed in this strain (Del Olmo *et al.* 2006), and at the behavioral level with the improved performance of LEW rats in the Morris water maze after cocaine self-administration (Del Olmo *et al.* 2007).

All these data suggest that the potentiation of presynaptic inputs to this region of the hippocampus could be operating during cocaine self-administration in LEW rats. The CA1 subfield is the primary output of the hippocampal circuit, and the collaterals of apical dendrites receive their main inputs from hippocampal subfield CA3 (Amaral & Witter 1989). The CA3 plays a central role in driving sharp wave-associated population events and the associated memory replay in CA1 (Csicsvari *et al.* 2000; Sullivan *et al.* 2011). Therefore, it is tempting to speculate that CA3–CA1 coupling could be involved in potentiating drug context memories during cocaine self-administration in the LEW strain. Moreover, the lack of this phenomenon in the F344 strain may be related to a protective role in the aforementioned differences in cocaine-induced reinstatement between these two strains of rats (Miguens *et al.* 2013). Underlying differences in the structural plasticity of this loop may also be implicated in the reported differences in spatial learning between these two genetically different strains of rats. Elucidating the presynaptic origin of cocaine-induced dendritic and spine plasticity in the hippocampus is of paramount importance and should be considered a priority for future studies.

Overall, the findings of this work suggest that genetic differences in cocaine-induced neural structural plasticity in the CA1 field of the hippocampus may be related to differences in the memory processing of drug reward cues that could explain the differential individual vulnerability to cocaine addiction.

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Conflict of Interest

The authors declare that they have no conflict of interest.

Authors Contribution

A. S., J. D., E. A. and M. M. were responsible for the study concept and design. S. M. C. contributed to the acquisition of animal data. A. S., A. K. and M. M. performed intracellular injections and imaging acquisition. A. S. and M. M. performed the morphometric analysis of the dendrites and spines, and data analysis. I. F. E., S. M. C. and A. K. assisted with data analysis and interpretation of findings. A. S. and M. M. drafted the manuscript. I. F. E., E. A. and J. D. provided critical revision of the manuscript for important intellectual content. All authors critically reviewed content and approved the final version for publication.

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